



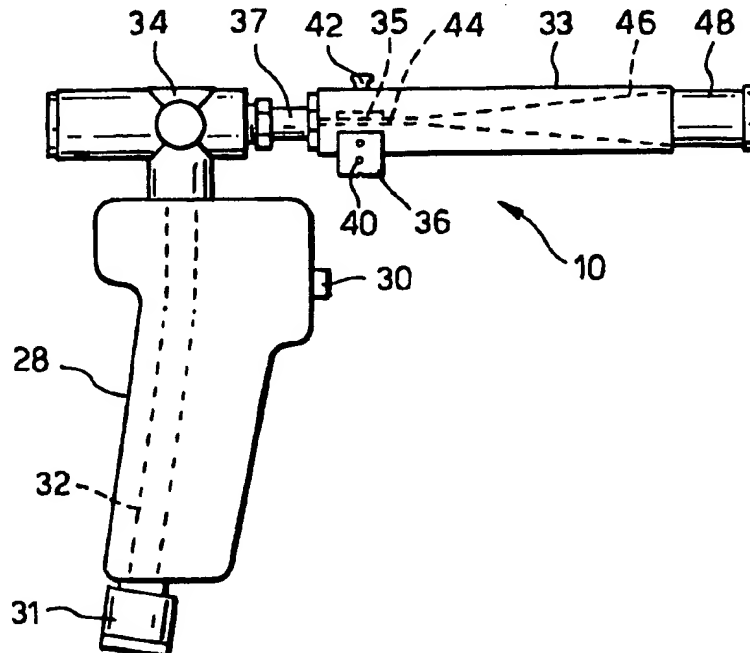
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(54) Title: **GAS-DRIVEN PARTICLE DELIVERY DEVICE**

(57) Abstract

A gas-driven particle delivery instrument is provided. The device includes various elements which enhance the utility and efficacy of the device, including a rotational flow element, a turbulent flow element, a flow constriction element, and combinations thereof.



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5 GAS-DRIVEN PARTICLE DELIVERY DEVICE

Technical Field

10 The present invention relates to the field
of delivering material into cells, more particularly
to instruments for delivering material into cells
using particle-mediated delivery techniques.

Background of the Invention

15 Particle-mediated delivery of materials,
particularly nucleic acid molecules, into living cells
and tissue has emerged as an important tool of plant
and animal biotechnology. Transient and long-term
expression of genetic material delivered via particle-
mediated techniques into target cells has been
20 demonstrated in a wide variety of microorganisms,
plants, and animals. Successful integration of DNA
into germ cells has also been demonstrated using these
techniques, and particle-mediated gene delivery
instruments have been used to deliver other materials
25 into cells, including pharmaceuticals and
biopharmaceuticals such as proteins, peptides and
hormones.

 As the fundamentals of the technology of
particle-mediated delivery have developed, attention
30 has increasingly shifted toward the development of
devices that offer the operator the ability to perform
the particle-mediated gene delivery in a swift and
convenient fashion. It is also desirable for the
operation of the delivery device to be efficient and
35 highly replicable.

One particular device, which uses compressed gas to accelerate carrier particles carrying biological materials into target tissue, is described in commonly owned International Publication No. WO 95/19799.

The distribution or spread of carrier particles delivered from a particle-mediated delivery device, such as the device of WO 95/19799, can be critical in some applications, particularly when biological material is being delivered, for example genetic material. In applications where germline transformation events are desired, the need to control the delivery pattern of carrier particles is substantially more acute than in other applications, such as where only transient expression of introduced genetic material is needed. When an infrequent germline transformation event is desired, it is generally necessary to uniformly accelerate the particles toward a large target area to increase the likelihood that one or more target cells will be transformed.

Accordingly, even though the device of WO 95/19799 and other related instruments have been suited for their intended purposes, there remains a need to provide for heightened uniformity and distribution of particles delivered from such devices.

Summary of the Invention

The invention is drawn to a gas-driven particle delivery device having elements which modify the flow of gas through the device. In one embodiment of the invention, a particle delivery device is provided which comprises a body having an acceleration passage formed therein. A rotational flow element is arranged within the acceleration passage and serves to impart a rotary motion in a flow of gas passing

therethrough prior to, or after entry of that gas flow into an acceleration chamber which forms a downstream part of the acceleration passage.

In various aspects of the invention, the rotational flow element is used to impart a rotational motion on the gas flow prior to, during, and/or after the gas flow has contacted particles which are to be delivered from the device. The rotational flow element can be any feature or structure disposed within an acceleration passage, which feature or structure is capable of imparting rotational motion on a gas flow passing therethrough. One particular rotational flow element comprises a plug or baffle which is arranged within the acceleration passage at a position upstream from a source of particles. Another rotational flow element comprises a structure, such as a plurality of thin propeller-like vanes, arranged within the acceleration passage at a position downstream from a source of particles.

In another embodiment, a particle delivery device is provided which comprises a body with an elongate acceleration chamber formed therein. The device includes a mixing chamber that communicates with the inlet of the acceleration chamber, and an upstream gas chamber that communicates with the mixing chamber. A rotational flow element is arranged within the upstream gas chamber, and imparts a rotary motion in a flow of gas passing from the upstream gas chamber into the mixing chamber to form a vortex within the mixing chamber.

In yet a further embodiment, a particle delivery device is provided which comprises a body with an elongate acceleration chamber formed therein. The device includes a source of particles that is arranged adjacent to an inlet for the acceleration chamber. A turbulent flow element is arranged

upstream from both the acceleration chamber and the source of particles, whereby such an element is used to create turbulence in a flow of gas passing therethrough prior to its contact with the source of particles. In one particular aspect of the invention, the turbulent flow element comprises a gas conduit arranged above the source of particles, wherein the gas conduit has a stepped portion of increased diameter.

10 In another embodiment of the invention, a particle delivery device is provided which comprises a flow constriction element that restricts flow of compressed gas into the device.

15 These and other objects, features and advantages of the present invention will become apparent from the following specification, read in light of the accompanying drawings.

Brief Description of the Drawings

20 Figure 1 is an illustration depicting the general operation of a gas-driven particle delivery device.

Figures 2A-2C are schematic illustrations of the effect of variations in the geometry of the exit nozzle in the device of Figure 1.

25 Figure 3 is a pictorial representation of a particle delivery device embodying the present invention.

30 Figure 4 is a cut-away view of a portion of the instrument of Figure 3 showing a rotational flow element disposed within a portion of the device.

Figure 5 is a side plan view of a rotational flow element according to the present invention.

35 Figure 6 is a cut-away, exploded view of a portion of a particle delivery device comprising a rotational flow element.

Figure 7 is a non-exploded view of Figure 6.

Figure 8 is a plan view of the upstream face of the rotational flow element of the device of Figure 6.

5 Figure 9 is a side plan view of the rotational flow element of the device of Figure 6.

Figure 10 is a cut-away view of a turbulent flow element according to the present invention.

10 Figure 11 is a plan view of a flow constriction element according to the present invention.

Figure 12 is a graphical representation of the particle delivery study described in Example 1.

15 Detailed Description of the Preferred Embodiment

Before describing the present invention in detail, it is to be understood that this invention is not limited to particular particle delivery devices or to particular carrier particles as such may, of course, vary. It is also understood that different
20 embodiments of the disclosed sample delivery modules and related devices may be tailored to the specific needs in the art. It is also to be understood that the terminology used herein is for the purpose of
25 describing particular embodiments of the invention only, and is not intended to be limiting.

It must be noted that, as used in this specification and the appended claims, the singular forms "a", "an", and "the" include plural referents
30 unless the content clearly dictates otherwise. Thus, for example, reference to "a particle" includes reference to mixtures of two or more particles, reference to "a therapeutic agent" encompasses one or more such agents, and the like.

35

A. Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. The following terms are intended to be defined as indicated below.

As used herein, the term "therapeutic agent" intends any compound or composition of matter which, when administered to an organism (human or animal) induces a desired pharmacologic, immunogenic, and/or physiologic effect by local, regional, and/or systemic action. The term therefore encompasses those compounds or chemicals traditionally regarded as drugs, vaccines, and biopharmaceuticals including molecules such as proteins, peptides, hormones, nucleic acids, gene constructs and the like.

More particularly, the term "therapeutic agent" includes compounds or compositions for use in all of the major therapeutic areas including, but not limited to, anti-infectives such as antibiotics and antiviral agents; analgesics and analgesic combinations; local and general anesthetics; anorexics; antiarthritics; antiasthmatic agents; anticonvulsants; antidepressants; antihistamines; anti-inflammatory agents; antinauseants; antineoplastics; antipruritics; antipsychotics; antipyretics; antispasmodics; cardiovascular preparations (including calcium channel blockers, beta-blockers, beta-agonists and antiarrhythmics); antihypertensives; diuretics; vasodilators; central nervous system stimulants; cough and cold preparations; decongestants; diagnostics; hormones; bone growth stimulants and bone resorption inhibitors; immunosuppressives; muscle relaxants; psychostimulants; sedatives; tranquilizers; proteins, peptides and fragments thereof (whether naturally

occurring, chemically synthesized or recombinantly produced); and nucleic acid molecules (polymeric forms of two or more nucleotides, either ribonucleotides (RNA) or deoxyribonucleotides (DNA) including both
5 double- and single-stranded molecules, gene constructs, expression vectors, antisense molecules and the like).

Particles of a therapeutic agent, alone or in combination with other drugs or agents, are
10 typically prepared as pharmaceutical compositions which can contain one or more added materials such as vehicles, and/or excipients. "Vehicles" and "excipients" generally refer to substantially inert materials which are nontoxic and do not interact with
15 other components of the composition in a deleterious manner. These materials can be used to increase the amount of solids in particulate pharmaceutical compositions. Examples of suitable carriers include water, silicone, gelatin, waxes, and like materials.
20 Examples of normally employed "excipients," include pharmaceutical grades of dextrose, sucrose, lactose, trehalose, mannitol, sorbitol, inositol, dextran, starch, cellulose, sodium or calcium phosphates, calcium sulfate, citric acid, tartaric acid, glycine,
25 high molecular weight polyethylene glycols (PEG), and combinations thereof. In addition, it may be desirable to include a charged lipid and/or detergent in the pharmaceutical compositions. Such materials can be used as stabilizers, anti-oxidants, or used to
30 reduce the possibility of local irritation at the site of administration. Suitable charged lipids include, without limitation, phosphatidylcholines (lecithin), and the like. Detergents will typically be a nonionic, anionic, cationic or amphoteric surfactant.
35 Examples of suitable surfactants include, for example, Tergitol® and Triton® surfactants (Union Carbide

Chemicals and Plastics, Danbury, CT),
polyoxyethylenesorbitans, e.g., TWEEN® surfactants
(Atlas Chemical Industries, Wilmington, DE),
polyoxyethylene ethers, e.g., Brij, pharmaceutically
5 acceptable fatty acid esters, e.g., lauryl sulfate and
salts thereof (SDS), and like materials.

When direct intracellular delivery is
intended, therapeutic agents (or pharmaceutical
preparations derived therefrom) can be coated onto
10 carrier microparticles using a variety of techniques
known in the art. Dense materials are preferred in
order to provide microparticles that can be readily
accelerated toward a target over a short distance,
wherein the microparticles are still sufficiently
15 small in size relative to the cells into which they
are to be delivered. It has been found that
microparticles having an average diameter of a few
microns can readily enter living cells without unduly
injuring such cells.

20 In particular, tungsten, gold, platinum and
iridium microparticles can be used as carriers for
therapeutic agents. Tungsten and gold are preferred.
Tungsten microparticles are readily available in
average sizes of 0.5 to 2.0 μm in diameter, and are
25 thus suited for intracellular delivery. Although such
microparticles have optimal density for use in
particle delivery methods, and allow highly efficient
coating with nucleic acids, tungsten may potentially
be toxic to certain cell types. Thus, gold is a
30 preferred material for use as carrier microparticles,
as gold has a high density, is relatively inert to
biological materials and resists oxidation, and is
readily available in the form of spheres having an
average diameter of from about 0.2 to 3 μm . Spherical
35 gold microparticles, or beads, in a size range of 1-3
microns have been successfully used in particle

delivery technologies, as well as gold provided in the form of a microcrystalline powder having a measured size range of about 0.2 to 3 μm .

5 B. General Methods

 In one embodiment, the invention is drawn to a component or topographical feature for use in a particle delivery device, which component or feature provides for an altered gas flow profile within the
10 device. The altered gas flow, in turn, provides for a dramatic improvement in particle distribution in directions lateral to the major axis of the gas flow. In another embodiment, a component or topographical feature is used in a particle delivery device for
15 providing a turbulent gas flow within the device. The turbulent flow allows for a more complete delivery of a payload of particles from the device. In yet a further embodiment, the invention is drawn to a means for limiting the amount of gas used to deliver
20 particles from a gas-driven particle delivery device, wherein such limitation serves to significantly reduce the audible report associated with a particle delivery operation without a concomitant reduction in the efficacy of particle delivery from the device.

25 Various particle delivery devices suitable for delivering a particulate therapeutic agent, or microparticles coated with a therapeutic agent, are known in the art, and are all suitable for use in conjunction with the present invention. Such devices
30 generally use a gaseous discharge to propel particles toward target cells. The particles can optionally be releasably attached to a movable carrier sheet, or removably attached to a surface along which a gas stream passes, lifting the particles from the surface
35 and accelerating them toward the target. Examples of gaseous discharge devices are described in U.S. Patent

No. 5,204,253 and in International Publication No. WO 95/19799.

Although the present invention is suitable for use with any particle delivery device, the invention is exemplified herein with reference to the device described in International Publication No. WO 95/19799. However, it is to be understood that any number of methods and devices similar or equivalent to those described herein can also be used in the practice of the present invention.

Turning now to the drawings, Figures 1 and 2 provide an illustration of the general method of operation of a particle delivery device such as that described in International Publication No. WO 95/19799. Components of the device are shown in slightly exploded view in some places for purposes of clarity. This particular depiction is intended to illustrate the basic operating principle of a particle delivery apparatus, rather than illustrate construction details.

Referring now to the device of Figure 1, a particle cartridge 14 is located within the particle delivery instrument. The particle cartridge 14 is an elongate concave or tubular structure that has a concave hollow passage passing through its center. A plurality of particles 16 are disposed on the interior of the cartridge. The particles, as discussed hereinabove, can be any particulate therapeutic agent or, preferably, can be comprised of small, dense carrier microparticles that are coated with a therapeutic agent, e.g., DNA or RNA, that is intended to be delivered into a target cell or tissue. Such microparticles may alternatively be coated with other types of biological materials such as peptides, cytokines, hormones, or proteins.

An actuator 18, for example a gas valve, gate, or rupturable membrane, is located upstream of the particle cartridge and is in fluid communication with the interior of the cartridge 14 via an appropriate conduit 17. The actuator is connected, by appropriate tubing generally indicated at 13, with a source of compressed gas 12. The source of compressed gas 12 can be a conventional commercial compressed gas tank, preferably containing an inert compressed gas such as helium. A reservoir of compressed gas is generally desirable between the gas source 12 and the actuator 18, however, the tubing 13 can function as such a reservoir.

Adjacent to the particle cartridge 14 is an orifice 20 which provides fluid communication with the interior of an acceleration chamber 22 which communicates, in turn, with a conical exit nozzle 24. The target, e.g., a patient, tissue surface, or cell, is designated as 19 in the Figure.

In general operation, the actuator 18 is used to release a pulse of compressed gas through the device. A particle acceleration passage, disposed between the actuator 18 and the exit nozzle 24, provides a path through which the released gas creates a gas stream traveling at significant speed. The gas stream accelerates through the particle acceleration passage and, as it passes through the interior of the particle cartridge 14, dislodges the particles 16. The accelerating gas stream, containing the dislodged particles, continues along the acceleration passage through the acceleration chamber 22, and into the exit nozzle 24.

One particularly important feature of the device of Figure 1 is the geometry of the exit nozzle 24. Referring now to Figure 2, three different possible geometries of the exit nozzle 24 are

illustrated schematically as Versions A, B, and C. Also depicted is the effect of these different exit nozzle geometries upon the delivery pattern of the particles 16. In Version A, the exit nozzle 24 does
5 not widen significantly toward the downstream end thereof. Thus, the exiting gas stream passes substantially linearly from the exit nozzle 24, and proceeds directly toward the target. As a result, the carrier particles continue in a relatively linear path
10 and provide a focused delivery pattern that impacts a relatively narrow area 25 of the target. While the particles 16 diverge somewhat from their linear flight, the divergence is quite small and insignificant.

15 In Version B of Figure 2, the exit nozzle 24 has an exceedingly wide angle of conical taper toward the downstream terminus thereof. In this configuration, the gas stream exits the instrument fairly linearly, and the particles 16 do not disperse
20 widely. Again, the particles impact a relatively compact portion 25 of the target.

A substantially different delivery pattern is obtained, however, when the angle of taper of the conical exit nozzle is less than a critical angle.
25 This phenomenon is illustrated as Version C in Figure 2. In particular, as the accelerated gas stream passes into the exit nozzle, it creates, through a vortex action, a vacuum between the route of passage of the gas stream and the sides of the exit nozzle 24.
30 This vacuum causes the gas stream to be pulled outwardly in all directions perpendicular to the direction of travel of the gas stream. In this manner, the gas stream and the particles entrained within the gas stream are dispersed in a direction
35 lateral to the major axis of the exit nozzle (i.e., the direction of travel of the particles). Thus, as

can be seen in Version C of Figure 2, the gas stream passing out of the instrument is spread laterally over a wider area, thereby distributing the particles 16 over a wider area and providing an improved delivery profile over the surface area 25 of the target. This avoids overdosing any one small area of the target with the delivered particles.

The exact angle of taper of the conical exit nozzle 24 can be varied to accommodate use of different gas pressures and relative sizes for the acceleration chamber 22. In an instrument which uses a commercial helium tank as the source of motive force, wherein the acceleration chamber 22 has a diameter of approximately 1/16 inch, an exit nozzle which tapers from 1/16 inch to 2/3 of an inch over a span of 3.3 inches will provide a satisfactory particle distribution pattern which covers a target surface having a diameter of from about 1/16 inch to about 2/3 of an inch. This represents over a 100-fold increase in the particle distribution pattern, with a concomitant 100-fold decrease in the particle distribution density.

In summary, then, the conical exit nozzle 24 of the device of WO 95/19799 can be configured significantly longer along its major axis than it is wide at either of its upstream or downstream termini in order to obtain a wider overall distribution of particles. In addition, by varying the pressure of the gas, the force with which particles impact the target 19 may be adjusted. At a minimum, however, the gas pressure provided by the source of motive force must be sufficient to dislodge the particles 16 from the cartridge 14. At the same time, the gas pressure should not be so great as to damage the target 19. When delivering coated carrier microparticles into intact animal skin using such devices, it has been

found that a discharged gas stream will not harm the targeted skin surface. At higher gas pressures, some minor reddening of the skin may occur, generally at tolerable levels. A regulated gas pressure, such as
5 that available from commercially available compressed helium tanks, has been found to be satisfactory for detaching the particles 16 from the cartridge 14, and delivering the same into epidermal cells of a target animal without untoward damage to the target skin or
10 cells. Lower pressures or higher pressures may work in particular applications, depending upon the density of the particles, the nature of the target surface, and the desired depth of particle penetration.

While use of the above-described exit nozzle
15 geometries and operation parameters provides for a significant distribution of delivered particles over a target surface, the distribution pattern is not as uniform as is desired. In particular, even though the particle distribution provided by the device of WO
20 95/19799 is better than that achieved with any other compressed gas-driven device, the pattern is still characterized by a concentration of particles impacted in the center of the target area, with a laterally decreasing distribution of particles extending from
25 that centralized area. Accordingly, it is a specific object of the invention to provide an element which serves to increase the uniformity of particle distribution obtainable from particle delivery devices.

30 In one particular embodiment of the invention, a rotational flow element is provided which can be positioned in a particle delivery device within an acceleration passage, for example at a location upstream from a source of particles (e.g., within the
35 fluid conduit 17 of the device of WO 95/19799), or at a location downstream from a source of particles

(e.g., within the acceleration chamber 22 of the device of WO 95/19799). The element can thus serve to re-channel all, or a portion, of the gas flow either prior to, or after its contacting the particles, thereby imparting a rotational motion on the gas flow prior to, during, and/or after the gas flow has contacted the particles. The element can be any feature or structure capable of imparting rotational motion on a gas flow. In one aspect of the invention, the element comprises one or more vanes, either disposed within, or depending from the interior surface of, a gas conduit within an acceleration passage. In one aspect of the invention, the vanes are positioned upstream of a source of particles to be delivered. In another aspect, the vanes are positioned downstream from a source of particles. The vane or vanes serve to re-channel at least a portion of the gas flow, forcing it to move or rotate about an axis. In yet another aspect, the element comprises a cylindrical plug or baffle disposed within a gas conduit that resides upstream from the source of particles. The plug or baffle contains one or more angled channels which allow an expanding flow of gas to pass therethrough so as to initiate a rotational flow in the expanding gas stream prior to, during, and/or after contact with the particles. These channels can be formed within the plug or baffle, disposed about the periphery of the plug or baffle such that a wall of the channel is provided by the gas conduit, or any combination of internal and peripheral channels can be employed. In any of such configurations, the rotational flow element serves to dramatically increase the lateral distribution of delivered particles, thus ensuring a more uniform particle distribution over the targeted area.

Not being bound by any particular theory, it is thought that imparting a rotation on the gas flow before, during, and/or after its contact with the particles assists in a turbulent intermixing of the particles within the gas, which in turn provides a better distribution of the particles within the expanding gas stream. Such rotational flow dynamics may also carry through to the exit nozzle of the particle delivery device, assisting in the formation of a laterally uniform dispersion of the delivered particles, possibly due to centrifugal forces. Regardless of the mechanism by which the result is achieved, the result is quite clear. The delivered particles are laterally dispersed from the major axis of the gas stream upon their exit from the particle delivery device, providing both a qualitatively and quantifiably measurable increase in the uniformity of particle distribution within a target area. In this manner, then, the present invention is broadly applicable for use in any gas-driven particle delivery device to provide enhanced uniformity in a particle distribution.

Referring to Figure 3, a particle delivery device similar to that of Figure 1 is generally indicated at 10. The device 10 comprises a handle 28 through which an inlet conduit 32 passes. The inlet conduit 32 terminates at one end with a coupler 31 which allows connection of the device 10 with an associated source of compressed gas. A trigger 30, situated on the handle 28, allows for actuation of the device by releasing a flow of gas into the device from the associated source.

An upstream gas conduit 37 connects the handle 28 with an elongate body 33, which body includes a cartridge chamber 35 capable of housing a particle cartridge. In the particular device of

Figure 3, a cartridge holder 36, mounted on the body 33, houses several particle cartridges which are configured as cylindrical tubes coated on their interior surfaces with particles for delivery from the device. In operation, cartridges from the cartridge holder are individually brought into position within the cartridge chamber 35 such that they are disposed within the path of a gas flow passing through an acceleration passage that extends from the upstream gas conduit 37 through an acceleration chamber 44. The acceleration chamber 44 terminates in an exit nozzle 46. The rotational flow element of the present invention is preferably situated within the upstream gas conduit 37 such that it may impart a rotational motion on the gas stream passing therethrough prior to contact of that gas stream with the particles in the cartridge chamber.

Referring now to Figures 4 and 5, one particular rotational flow element is shown which comprises a baffle 50 having an upstream face 52 and a downstream face 54. The baffle is configured as a plug which can be inserted within the upstream gas conduit 37 at a location adjacent to the cartridge chamber 35. The baffle 50 includes one or more gas channels 56 disposed in a radial array about its periphery. The baffle can be comprised of any suitably resilient material which is either machinable or molded, for example, metals, metal alloys and rigid polymeric materials. The gas channels extend along the length of the baffle in a direction that is substantially in the direction of gas flow through the gas conduit. However, as can be seen with particular reference to Figure 5, each channel can be canted or angled at a defined angle θ relative to the major axis of the baffle.

The particular angle θ can vary over a range of about 0-15°, and is preferably in the range of about 0-11°. A small angle θ of about 0-5° provides for deeper penetration of particles delivered from the particle delivery device. A medium angle θ of about 7-11° provides the broadest distribution of particles in a direction lateral to the direction of gas flow through the particle delivery device.

The baffle 50 can comprise external threads which cooperate with corresponding threading within the gas conduit 37, or the baffle can have a substantially smooth external surface, for example where the baffle is compression fit within the gas conduit. In operation, a flow of gas released within the particle delivery device passes into the gas conduit 37 where it contacts the upstream face 52 of the baffle 50. The expanding gas flow is then caused to pass through the gas channels 56, which imparts a rotational motion on the gas flow proportional to the angle θ . This rotational gas flow then travels into a particle cartridge where it picks up the particles from the interior surface of the cartridge for delivery to a target surface.

Referring now to Figures 6-9, a related rotational flow element baffle is generally indicated at 70. The baffle 70 is arranged within a particle delivery device between an upstream chamber 72 which provides the initial chamber into which compressed gas is released, and a downstream mixing chamber 74. The baffle 70 has an upstream face 76, a downstream face 78, and an outer surface 80. A linear central bore 82 extends between the upstream and downstream faces 76 and 78, wherein the central bore is coaxial with the major axis of the baffle 70. An annular seat 84 in the upstream face 76 provides a recessed fitting which accepts and retains a cylindrical particle cartridge

86. The annular seat 84 surrounds and is coaxially aligned with the central bore 82.

The particle cartridge 86 has a plurality of particles 88 coated on the interior surface thereof.

5 As can be seen with reference to Figures 6 and 7, the particle cartridge 86 sits within the annular seat 84 and projects into the upstream chamber 72. Referring now to Figures 8 and 9, the baffle 70 has one or more gas channels 90 in the outer surface 80 thereof,
10 wherein the gas channels are disposed in a radial array about the central bore 82. As described hereinabove, the gas channels can be canted or angled relative to the major axis of the baffle 70 in order to impart a rotational motion on gas flowing through
15 the particle delivery device. The relative angle of the gas channels can range between 0-15° depending on the amount of rotational motion that is desired for the gas flow.

In operation, a flow of gas released into
20 the upstream chamber 72 travels toward the upstream face 76 of the baffle 70, with a portion of the gas flow entering the particle cartridge 86. The reduced diameter of the central bore 82 relative to the diameter of the annular seat 84 and hence the particle
25 cartridge 86, restricts the amount of gas that can flow through the particle cartridge to a fixed percentage of the total gas flow. The bulk of the gas flow thus travels around the outer surface of the baffle 70 and through the gas channels 90. This
30 induces the formation of a vortex at a central point within the mixing chamber 74 where the gas flowing through the gas channels converges. An axial beam of particles 88 which have been dislodged from the interior surface of the particle cartridge 86 travels
35 through the central bore 82 of the baffle in a substantially linear direction, and is thus delivered

into the center of the vortex formed within the mixing chamber. As the vortexing gas flow and particle beam enter the acceleration chamber 92, the spinning gas flow contacts the axial beam of particles which are
5 then accelerated and centrifuged to impart a final trajectory as they pass through the nozzle 94, distributing the particles uniformly over a target area.

Here again the baffle 70 can be comprised of
10 any suitable machinable or moldable material that can withstand the force of a compressed gas flow suitable for delivery of particles through the particle delivery device. The baffle 70 can further include external threads on the outer surface 80 thereof in
15 order to facilitate coupling to the upstream chamber 72.

The rotational flow elements of the present invention serve to impart a rotational component in the flow of compressed gas stream passing through the
20 particle delivery device. This rotational flow component causes some surprising results in the operation of particle delivery devices. One unexpected result is that a device fitted with a rotational flow element, referred to here sometimes as
25 a "spinner" instrument, is more effective in picking up particles from the interior of the particle cartridge 14. However, the rotational flow elements of the present invention provide their greatest benefit by substantially enhancing uniformity in
30 particle distribution over a target area.

In another embodiment of the invention, a turbulent flow element is provided which can be positioned in a particle delivery device at a location upstream from a source of particles (e.g., within the
35 fluid conduit 17 of the device of WO 95/19799). The element serves to perturb all, or a portion, of the

gas flow prior to its contacting the particles, thereby improving the reliability of particle release from the particle source. The turbulent flow element can thus be any feature or structure capable of

5 disrupting a gas flow in such a way as to impart a flow turbulence. In one aspect of the invention, the element comprises one or more topographical features disposed on the interior surface of an upstream gas chamber. Such features can comprise a ridge,

10 shoulder, groove, corrugation, or any combination thereof, which features serve to disrupt or perturb the flow of gas passing through a chamber or conduit. In a particular aspect of the invention, the turbulent flow element comprises a small step in the interior

15 surface of a cylindrical gas chamber which establishes a chamber area of slightly increased diameter bounded by areas of lesser diameters, through which areas an expanding gas flow passes prior to contacting a source of particles.

20 Referring now to Figure 10, a turbulent flow element is generally indicated at 100. The flow element 100 is adapted for insertion into a particle delivery device, wherein it accepts a released gas flow through an upstream opening 102 which has a first

25 diameter A. As the gas flow proceeds through the element, it enters a stepped portion 104 having a slightly greater diameter B. The stepped portion of the element 100 extends along a substantial portion of the overall length of the element and is bounded on

30 its downstream side with a downstream opening 106 having a diameter A equal to that of the upstream opening. The stepped portion 104 of the element is sufficient to introduce a turbulence in the gas flow prior to its contact with a source of particles

35 arranged adjacent to the downstream opening 106. This turbulence improves the reliability of particle

release from the particle source, improving particle delivery efficiency. In a particular embodiment, the diameter A of the upstream and downstream openings 102 and 106 is about 0.250 inches, and the diameter B of the stepped portion 104 is about 0.280 inches, providing a step of about 0.03 inches.

In yet a further embodiment of the invention, a flow constriction element is provided for use in a particle delivery device. Referring to Figure 3, the constriction element is configured for insertion into the particle delivery device adjacent to the point of coupling between the device and the associated source of compressed gas. In particular, the constriction element can be inserted at the junction between the inlet tube 32 and the connector 31. The constriction element is generally comprised of a disk of flexible or resilient material sized to restrict passage of gas into the delivery device to a small orifice. The orifice can be quite small, for example, an orifice of about 200 to 250 μm has been found to be sufficient. Referring now to Figure 11, a flow constriction element constructed according to the present invention is generally indicated at 60. The element is configured as a disk having an orifice 62 passing therethrough.

The purpose of the restriction provided by the orifice 62 is to isolate an aliquot of compressed gas in the instrument for each particle delivery operation. With particular reference to the device described in International Publication No. WO 95/19799, the source of compressed gas supplies additional compressed gas through the instrument at all times, not just during delivery operations. As it turns out, more gas is delivered into the instrument upon actuation than is actually required for effective delivery of the particles to a target surface. In

fact, the excess gas travelling through the instrument merely adds to the impact of the delivery operation on the target without providing a corresponding benefit. By providing the flow constriction element 60 in the device, the compressed gas bleeds through the orifice into the interior of the gene delivery instrument between delivery operations. When the device is actuated, then, there is a charge or aliquot of compressed gas already present in the instrument itself, occupying the various chambers and conduits within the device. This charge of compressed gas is, as it turns out, sufficient to deliver the particles from the particle source to the target surface. When the charge of compressed gas has been thus released, the flow restriction provided by the flow constriction element 60 prevents an additional volume of compressed gas from flowing through the instrument. After the delivery operation, the compressed gas will again recharge the instrument until a pressure equilibrium is reached.

In other related aspects of the invention, the concept of providing a single aliquot (charge) of gas within the particle delivery device to discharge a payload of particles can be carried out using other mechanisms. For example, instead of using a flow constriction element such as the element 60, a combination of valves can be used to achieve the same effect. In one particular arrangement, an inlet valve can be provided which is closed during operation of the instrument, and then opened when the instrument is not being operated in order to charge the instrument for the subsequent operation. For example, if an electric or solenoid operated valve is used as the main valve for the instrument, two valves can be operated alternately wherein an inlet valve is closed

whenever the main valve is opened, and the inlet valve is opened whenever the main valve is closed.

The benefits of the flow constriction element are several fold. For example, the audible report created by operation of the instrument is dramatically reduced. At 500 psi of compressed gas, a typical particle delivery device will generate an approximately 103 dB report upon discharge, whereas the same instrument having the present flow constriction element generates only an 88 dB report upon discharge. There is also a perceptible dampening of the sensory feel of the gas discharge from a particle delivery device when the present constriction element is employed. Furthermore, there is less damage to sensitive target tissues or cells when the flow constriction element is used.

It is specifically intended herein that the various embodiments of the invention can be used alone or in any combination. In this regard, each embodiment is capable of independently providing a unique and advantageous improvement in the performance of a particle delivery device. However it is particularly advantageous to use both a rotational flow element and a flow constriction element to obtain optimum results.

C. Experimental

Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperatures, etc.), but some experimental error and deviation should, of course, be allowed for.

Example 1Particle Delivery Distribution

In order to assess the effect of a rotational flow element on the performance of a particle delivery device, the following study was carried out. A particle delivery device, such as the "gene gun" described in International Publication No. WO 95/19799, was used to deliver gold microparticles into a Parafilm® block covered by a Mylar® film. This arrangement approximates a typical skin tissue target surface, and is sufficiently opaque to allow visual and/or optical assessments of the particle distribution in a target area. In the present study, a Model GS300 optical reader (Hoefer Scientific) was used to generate a graphical representation of the distribution of the gold microparticles in a transect across the center of the particle spread.

Six different particle deliveries were conducted, using identical particle payloads which were delivered from an ACCELL® particle delivery device (PowderJect Vaccines, Madison, WI) operated at 400 psi helium. Each delivery was conducted using a rotational flow element that provided a different degree of spin (0°, 2°, 3.5°, 5°, 7°, and 11°).

The results of the study are presented in the graph of Figure 12. In the graph, the ordinate value represents particle density, while the abscissa value represents particle spread. As can be seen in Figure 12, the curve A (obtained from the particle delivery using a rotational flow element that provides 0° spin) is both tall and narrow, demonstrating that the particles did not spread laterally and were concentrated in the center of the target area. In contrast, the curve C (obtained from the particle delivery using a rotational flow element that provides 3.5° spin) is much wider relative to curve A,

indicating a higher particle spread over the target area. In addition, curve C is lower, indicating that there is not a particularly high concentration of particles in the center of the target area. The curve
5 F (obtained from the particle delivery using a rotational flow element that provides 11° spin) shows that this higher degree of rotation significantly disperses the particles over the entire target area.

Subsequent gene delivery experiments into
10 the skin of animals confirms that the rotational flow element improves operation of particle delivery devices. The use of the element was shown to provide comparable levels of reporter gene expression in test animals when compared with parallel experiments using
15 devices without the flow elements. In addition, it was observed that the occurrence of erythremia in the skin of test animals was significantly reduced when using the rotational flow element in the particle delivery devices.

20 Accordingly, novel gas flow modifying elements for use with particle delivery devices have been described. Although preferred embodiments of the subject invention have been described in some detail, it is understood that obvious variations can be made
25 without departing from the spirit and the scope of the invention as defined by the appended claims.

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We claim:

1. A device for delivering particles into a target cell or tissue, said device comprising:
 - 5 a body having an acceleration passage formed therein, said passage having an inlet and an outlet; and
 - a rotational flow element arranged within the acceleration passage, wherein said rotational flow
 - 10 element imparts a rotary motion in a flow of gas passing therethrough.
2. A device for delivering particles into a target cell or tissue, said device comprising:
 - 15 a body having an acceleration passage, said passage having an inlet and an outlet;
 - an actuator for admitting a gaseous flow through the inlet into the passage, the gaseous flow accelerating through the passage and carrying
 - 20 particles out of the outlet; and
 - a rotary flow inducing element located in the passage to impart a rotary motion to the gaseous flow.
- 25 3. A device for delivering particles into a target cell or tissue, said device comprising:
 - a body having an elongate acceleration chamber formed therein, said chamber having an inlet and an outlet; and
 - 30 a rotational flow element arranged upstream from the inlet of the acceleration chamber, wherein said rotational flow element imparts a rotary motion in a flow of gas passing therethrough prior to entry of said gas flow into the acceleration chamber.

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4. The device of claim 3, wherein said rotational flow element comprises a baffle disposed within a gas chamber or conduit arranged upstream from the acceleration chamber, said baffle having an upstream face and a downstream face.

5. The device of claim 4, wherein said baffle is comprised of a substantially cylindrical plug having an outer surface with a gas channel formed therein, said gas channel allowing passage of a gas flow from the upstream face to the downstream face of the baffle.

6. The device of claim 5, wherein the gas channel is arranged in angular relation with the major axis of the baffle.

7. The device of claim 5 further comprising a plurality of gas channels arranged in a radial array about the outer surface of the baffle, wherein said gas channels are each arranged in angular relation with the major axis of the baffle and allow passage of a gas flow therethrough.

8. The device of claim 5, wherein said baffle further comprises a substantially linear central bore passing between the upstream and downstream faces of the baffle, said central bore allowing passage of a gas flow therethrough.

9. The device of claim 8, wherein said baffle further comprises an annular seat coaxially aligned with the central bore and disposed within the upstream face of the baffle.

10. The device of claim 9, wherein the annular seat is configured to accept and retain a cylindrical cartridge containing particles to be delivered from the device.

5

11. A device for delivering particles into a target cell or tissue, said device comprising:

a body having an elongate acceleration chamber formed therein, said chamber having an inlet,
10 and an outlet which terminates in an exit nozzle;

a mixing chamber having an inlet, and an outlet that communicates with the inlet of the acceleration chamber;

an upstream gas chamber having a outlet that
15 communicates with the inlet of the mixing chamber, wherein a rotational flow element is arranged in the outlet of the upstream gas chamber and imparts a rotary motion in a flow of gas passing from the upstream gas chamber into the mixing chamber.

20

12. The device of claim 11, wherein said rotational flow element comprises a substantially cylindrical baffle having an outer surface, an upstream face and a downstream face, said baffle
25 further having a plurality of gas channels formed in the outer surface thereof which allow passage of a gas flow from the upstream face to the downstream face of the baffle.

30

13. The device of claim 12, wherein flow of gas through the plurality of gas channels in the baffle creates a vortex within the mixing chamber.

14. The device of claim 13, wherein the
35 baffle further comprises a substantially linear central bore passing between the upstream and

downstream faces of the baffle, said central bore allowing a gas flow containing particles to pass therethrough.

5 15. A device for delivering particles into a target cell or tissue, said device comprising:

 a body having an elongate acceleration chamber formed therein, said chamber having an inlet and an outlet;

10 a source of particles to be delivered from the device, wherein said source is adjacent to the inlet of the acceleration chamber; and

 a turbulent flow element arranged upstream from the inlet of the acceleration chamber and the
15 source of particles, whereby said turbulent flow element creates turbulence in a flow of gas passing therethrough prior to contact of said gas flow with the source of particles.

20 16. The device of claim 15, wherein the turbulent flow element comprises a gas conduit having a stepped portion of increased diameter.

 17. A device for delivering particles
25 into a target cell or tissue, said device comprising:

 a body having an elongate acceleration chamber formed therein, said chamber having an inlet and an outlet;

 a source of compressed gas coupled to the
30 body for delivery of a gas flow into the inlet of the acceleration chamber; and

 a flow constriction element that limits flow of gas from the source into the body.

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18. The device of claim 17, wherein the flow constriction element comprises a plug having an orifice passing therethrough.

5 19. The device of claim 17 further comprising a rotational flow element arranged upstream from the inlet of the acceleration chamber, wherein said rotational flow element imparts a rotary motion
10 in a flow of gas passing therethrough prior to entry of said gas flow into the acceleration chamber.

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Fig.1.

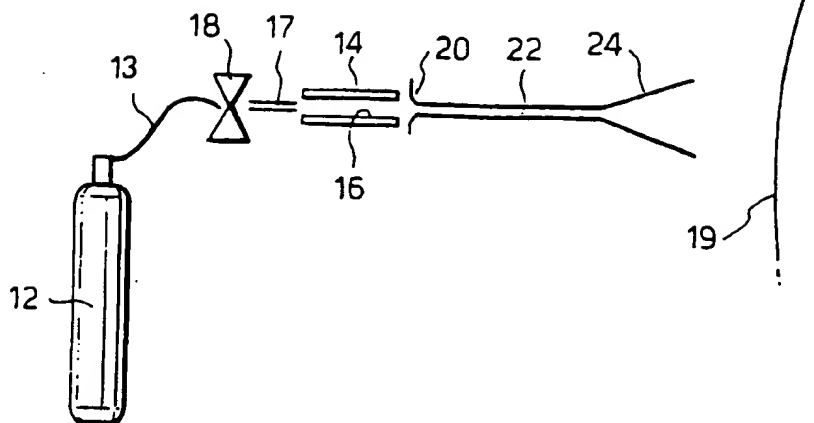


Fig.2A.

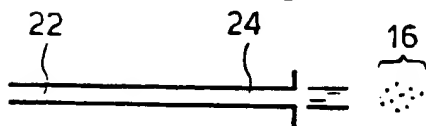


Fig.2B.

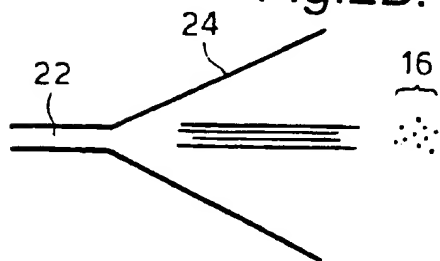
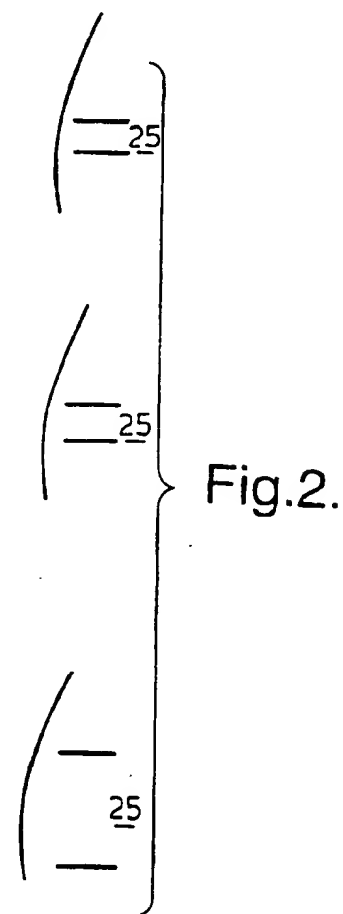
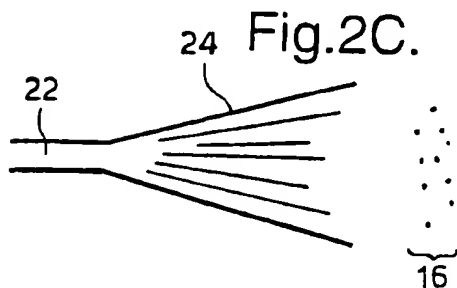


Fig.2C.



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Fig.3.

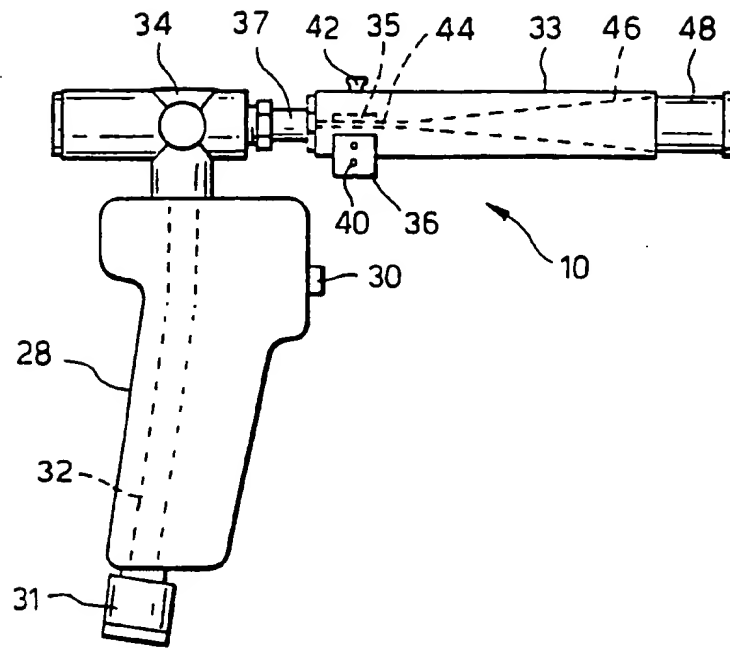


Fig.4.

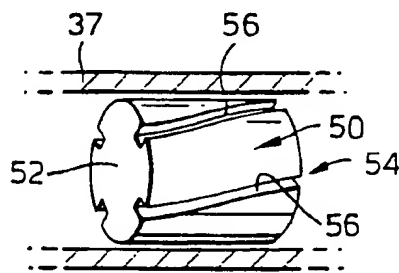
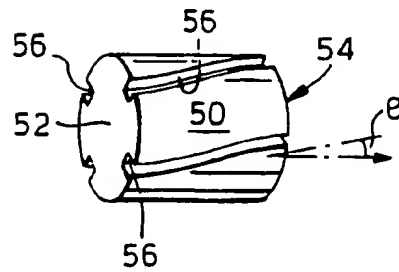


Fig.5.



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Fig.6.

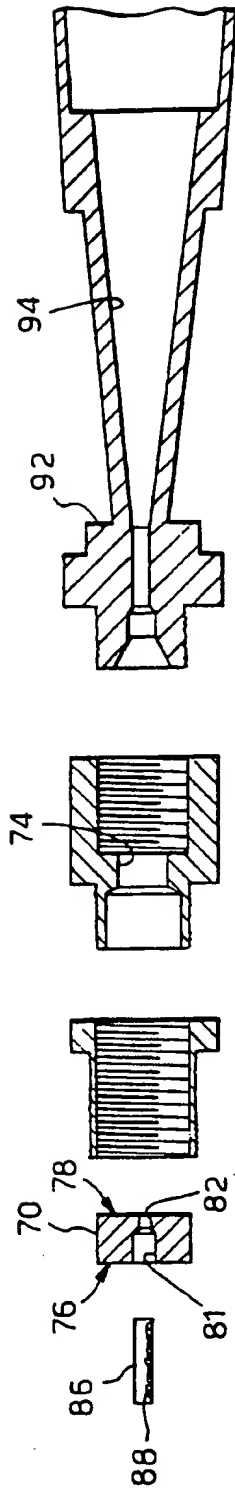


Fig.7.

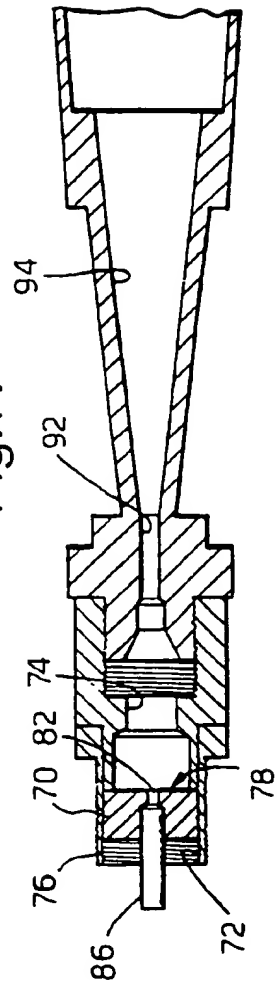


Fig.8.

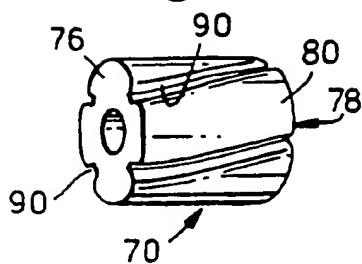


Fig.9.

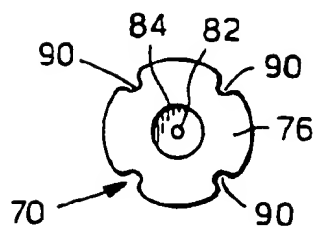


Fig.10.

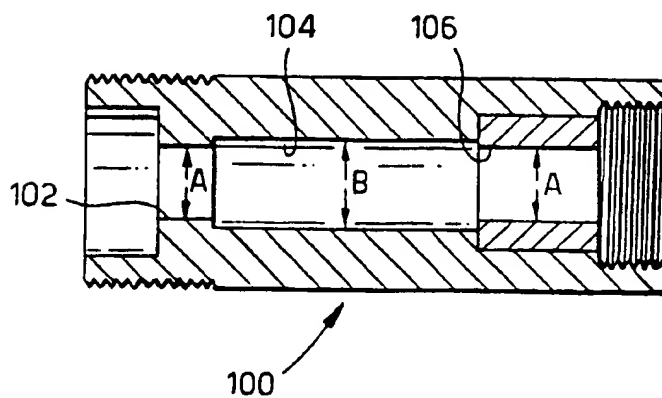
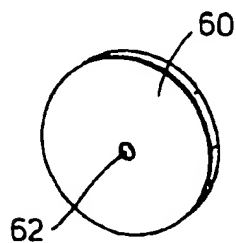
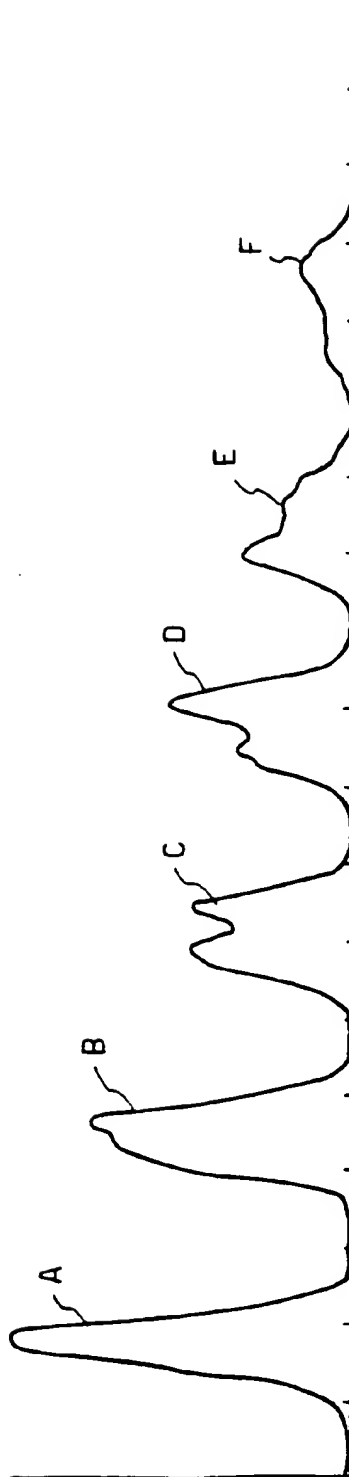


Fig.11.



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Fig.12.



INTERNATIONAL SEARCH REPORT

Int. (ional Application No
PCT/US 97/17202

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12M3/00 B24C5/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12M C12N B24C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	DE 44 04 954 A (FASTJE HELMUT) 31 August 1995	1-8,
Y	see column 1, line 52 - line 62	11-15, 17
	see column 1, line 15 - line 17; claims; figures	1, 11, 15, 17
	see column 1, line 29 - line 32	
	see page 6, line 17 - line 18	

X	US 4 521 996 A (GRUND PETER ET AL) 11 June 1985	1-5,
Y	see column 1, line 35 - line 52; claims; figures	11-13,
	---	15-17
Y	WO 95 19799 A (AGRACETUS) 27 July 1995 cited in the application	1, 11, 15, 17
	see the whole document	

	-/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

16 February 1998

Date of mailing of the international search report

23/02/1998

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 97/17202

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 691 183 A (HARTMANN KULBA BAUCHEMIE GMBH) 10 January 1996	1-4, 11-13, 15
Y	see claims: figures -----	1, 11, 15, 17

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/US 97/17202

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
DE 4404954	A	31-08-1995	NONE	
US 4521996	A	11-06-1985	DE 3309941 A EP 0119399 A JP 59187466 A	20-09-1984 26-09-1984 24-10-1984
WO 9519799	A	27-07-1995	AU 674815 B AU 1729595 A BR 9505691 A CA 2158733 A CN 1124460 A EP 0690732 A JP 8509131 T US 5584807 A	09-01-1997 08-08-1995 16-01-1996 27-07-1995 12-06-1996 10-01-1996 01-10-1996 17-12-1996
EP 0691183	A	10-01-1996	NONE	